Fluorescence dissolved organic matter (fDOM) analysis of inhalant and exhalent water samples from sponges studied at an artificial reef off the coast of the Florida Keys, USA in August 2021

Website: https://www.bco-dmo.org/dataset/906070
Data Type: Other Field Results, experimental
Version: 1
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Project
» Collaborative Research: Investigations into microbially mediated ecological diversification in sponges (Ecological Diversification in Sponges)

Contributors	Affiliation	Role
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Abstract
Coral reefs are biodiverse and productive ecosystems that are found in typically oligotrophic (low nutrient) environments. The focus of research on nutrient acquisition and partitioning among coral reef organisms has, historically, been focused on corals and their symbiotic zooxanthellae. For example, different clades of zooxanthellae are adapted to different irradiance levels which facilitates coexisting species of corals. Comparatively very few studies have asked if and how coexisting sponges and their symbiotic microbial communities partition nutrients (i.e., utilization of different nutrient pools across species). To address this question, this study set up an artificial reef off the coast of the Florida Keys using dominant emergent sponges found in the Caribbean and Florida Keys. Inhalant and exhalent water samples were collected using Vacusip. Samples were filtered with a 0.2-micrometer supor filter and then processed for dissolved nutrients using fluorescence dissolved organic matter (fDOM). This is the first application of fDOM analysis to sponges. In this study, we found that the microbial abundance (i.e. the commonly used high and low microbial abundance (HMA/LMA) classification) was not an effective indicator for the way in which nutrients are processed by these sponge species. Additionally, the use of fDOM analysis indicated nuance in DOM utilization across species with differential consumption of fDOM components across sponge species. In summary, rather than microbial abundance alone, a combination of sponge species identity and the composition of the symbiotic microbial community members (e.g., presence of photosymbionts) appears to explain the most variation in nutrient processing by sponges. These results provide the first support for resource partitioning of dissolved nutrients across coexisting sponge species and provide support for the evolutionary importance of microbial communities in sponges.

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Coverage

Spatial Extent: **Lat**:24.562003 **Lon**:-81.408711
Temporal Extent: 2021-08-04 - 2021-08-12

Methods & Sampling

This study was conducted at an artificial reef near Looe Key reef in the Florida Keys (USA), depth 10 meters. The artificial reef was removed after the experiment was completed.

To examine differences in nutrient profiles across sponge species, an artificial reef was constructed with 5 rows, with 10 cinder blocks per row, the rows were arranged in a semi-circle, and rebar was run through each individual row and secured at the middle and ends to ensure that the rows would not move over the duration of the experiment. Species (n = 10 replicates of 10 species) were placed on the rows in roughly a Latin-square design to spread out any environmental variable that may exist on the artificial reef across replicates of species. The depth of the reef was approximately 7 meters and immediately surrounding the reef was sand on all sides. The rows of sponges were placed so that they were as equal as possible in exposure to light and water flow. Two pieces of 5-millimeter (mm) polystyrene mesh were placed on top of each of the cinder blocks and secured with cable ties. Ten individuals of each of the 10 focal species (*Aiolochroia crassa*, *Aplysina cauliformis*, *Aplysina fulva, Amphimedon compressa*, *Iotrochota birotulata*, *Ircinia felix*, *Niphates digitalis*, *Callyspongia aculeata*, *Verongula rigida*, and *Xestospongia muta*) were selected and individuals were attached to the polystyrene mesh using cable ties. The sponges were allowed to acclimate for 4 months. The sponge species were selected based on prevalence in the Caribbean and included four low microbial abundance (LMA) species (*Amphimedon compressa*, *Iotrochota birotulata*, *Niphates digitalis*, *Callyspongia aculeata*) while the remaining six species are considered high microbial abundance (HMA) sponges.

In Situ Water collection:
Samples for all nutrient analyses were collected using a modular vacuum setup (VacuSIP) which was implemented and modified from Morganti et al. 2016. The VacuSIP included poly ether ether ketone (PEEK) tubing (used commonly in HPLC instruments) that was placed over the sponge osculum (sponge exhalent seawater) or near the sponge (inhalant seawater) and positioned using tripods. The tubing was connected via syringe needle to pressurized 250 milliliter (mL amber glass bottles with Teflon septate caps. The 250 mL glass vials were pre-combusted within days of sampling (6 hours at 450 °Celsius) and pressurized manually to -15 psi and this pressure increased slightly at the depth of the artificial reef. VacuSIP lines were acid rinsed in 10% HCl. Pumping was confirmed using fluorescent dye before each collection and the dye was allowed to clear before sampling. Tubing was then positioned directly above the pumping oscula for small sponges or inside the oscula close to the sponge for larger sponges. VacuSIP lines were then attached to the appropriate 250 mL bottles in the crates by sticking the needles at the end of the line into the septa of the bottle. Tubing for inhalant water collection was inserted into the appropriate 250 mL bottles. The apparatus contained fewer lines than 250 mL collection bottles, so the apparatus was set up to fill half of the bottles in the collection crate for 120 minutes, then the lines were moved to new bottles to fill the remaining bottles for 90 minutes. Once at the surface, the 250 mL bottles for each individual sample were combined into 2 liter (L) bottles, one for inhalant and one for exhalent water samples, that were labeled and then stored on ice in coolers until they were filtered.

Water Filtration:
A Cole-Palmer Masterflex L/S Intertek fitted with a Masterflex L/S easyload II head and Multichannel Pump Head Cartridges was used to filter the samples at a rate of 40 mL per minute through High-Performance Precision Pump Tubing, PharMed® BPT, L/S 15 with a 0.2 micrometer (µm) Supor filter into 1L acid-rinsed polycarbonate bottles that were covered with aluminum foil. The filters were archived for future microbiome analysis and the filtrate was stored in 40 mL amber vials with Teflon septa. Filtrate for dissolved organic carbon (DOC) and total dissolved nitrogen (TDN) was acidified to ~pH 2 using 6N HCl and stored at 4°Celsius. Samples for DOC and TDN were sent to the UGA Stable Isotope Ecology Laboratory for analysis using a Shimadzu TOC-5000A Total Organic Carbon Analyzer. While DOC and TDN samples were collected, there was contamination in these samples from an unknown source and therefore, DOC and TDN were not used in any data analysis. Filtrate for fluorescent dissolved organic matter (fDOM) was not acidified and stored at 4°Celsius. The fDOM samples were shipped to a collaborator at the University of Hawaii (Dr. Craig Nelson) where they were stored until analysis.

The investigator worked with the Nelson lab at UH to process and analyze the fDOM as samples described below.

fDOM Sample Processing:
Following the methods from Nelson et al. (2015), samples were analyzed with a Horiba Aqualog scanning fluorometer with 150 watts Xe excitation lamp, Peltier-cooled CCD emission detector, and simultaneous absorbance spectrometer. Quartz cuvettes of 1-centimeter (cm) diameter, which were DIW-leached and rinsed, were used to measure fluorescence. Samples were brought to room temperature while the Xe bulb warmed up.

Known problems or issues:
Not all replicates of each sponge were sampled. Dissolved Organic Carbon (DOC) and Total Dissolved Nitrogen (TDN) values are absent from the dataset due to contamination of samples.

Percent change in nutrients was calculated only for fDOM components, not for inorganic nutrients or for particulates captured on the GF/F filters.

Several samples were not analyzed for fDOM or for inorganic nutrients (broken or lost), and only a subset of samples were analyzed with the GF/F filters.

Data Processing Description

Fluorescent dissolved organic matter (fDOM) processing:
Excitation-emission matrices (EEMs), 3D contour plots of excitation and emission fluorescence, were measured from the 108 samples, starting with 4 DIW filled cuvettes as blanks at the beginning and end of the analysis. Scans were processed using a MATLAB script that use parallel factor analysis (PARAFAC) to identify peaks in the EEMs that correspond to previously characterized fluorescent components of humic-like and amino acid-like components (https://github.com/zquinlan/fDOMmatlab/script.md) (Coble, 1996; Nelson et al 2015).

Inorganic nutrient processing:
Filtrate (0.2 µm) was stored in acid-cleaned polypropylene bottles at -20° Celsius for inorganic nutrient analysis. Samples were shipped to Oregon State University (OSU) nutrient analysis laboratory. At OSU, Technicon AutoAnalyzer II™ components were used to measure phosphate and ammonium; and Alpkem RFA 300™ components were used for silicic acid, nitrate plus nitrite, and nitrite. Gordon et al. (1994) described analytical methods and data processing. The phosphate method was a modification of the molybdenum blue procedure of Bernhardt and Wilhelms (1967), in which phosphate is determined as reduced phosphomolybdic acid employing hydrazine as the reductant. The nitrate + nitrite analysis used the basic method of Armstrong et al. (1967), with modifications to improve the precision and ease of operation. Sulfanilamide and N-(1-Napthyl)ethylenediamine dihydrochloride react with nitrite to form a colored diazo compound. For the nitrate + nitrite analysis, nitrate was first reduced to nitrite using an OTCR and imidazole buffer as described by Patton (1983). Nitrite analysis was performed on a separate channel, omitting the cadmium reductor and the buffer. The method was based on that of Armstrong et al. (1967) as adapted by Atlas et al. (1971). Addition of an acidic molybdate reagent forms silicomolybdic acid which is then reduced by stannous chloride. This indophenol blue method is modified from ALPKEM RFA methodology which references Methods for Chemical Analysis of Water and Wastes, March 1984, EPA-600/4-79-020, "Nitrogen Ammonia", Method 350.1 (Colorimetric, Automated Phenate). All values are concentration in micromoles per liter of seawater.

Elemental analysis of particulates retained on glass fiber filters:
Combusted glass fiber filters (GF/F, 0.7 µm mesh, 13 mm diameter) were weighed prior to water filtration, then oven-dried at 60° Celsius for 24 hours before weighing again after water filtration. Dried filters were carefully packed into tin capsules (8 x 5 mm, EAconsumables), stored in a 96-well plate and shipped to the University of Georgia (UGA) Stable Isotope Ecology Laboratory (SIEL) for elemental analysis. Blank combusted filters were also weighed and added to the plate as background controls. At UGA, samples were analyzed for carbon and nitrogen content using a 2 Thermo Flash 1000 series elemental analyzer. Elemental standards included the National Institute of Standards and Technology (NIST) standard reference material 1570a. The percent carbon and nitrogen content were calculated for each sample and obtained from UGA SIEL.

BCO-DMO Processing Description

- Imported original file named "fDOM_and_nutrients_combined_ForBCODMO_ArtificialReef2021_Fiore.xlsx" into the BCO-DMO system.
- Renamed fields to comply with BCO-DMO naming conventions (replaced spaces with underscores and removed or replaced other non-allowed characters).
- Rounded columns to 5 decimal places.
- Named the final file "906070_v1_artificial_reef_fdom.csv".

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Data Files

File
906070_v1_artificial_reef_fdom.csv (Comma Separated Values (.csv), 33.56 KB) MD5:8ec9b6de36bd9cc0718a4f4ad5f2419c Primary data file for dataset ID 906070, verison 1.

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Related Publications

Armstrong, F. A. J., Stearns, C. R., & Strickland, J. D. H. (1967). The measurement of upwelling and subsequent biological process by means of the Technicon Autoanalyzer® and associated equipment. Deep Sea Research and Oceanographic Abstracts, 14(3), 381–389. doi:10.1016/0011-7471(67)90082-4
Methods

Atlas, E. L., Hager, S. W,, Gordon, L. I., & Park, P. K. (1971). A practical manual for use of the Technicon Autoanalyzer in sea water nutrient analyses. Oregon State University, Department of Oceanography. Technical report.
Methods

Bernhardt, H., and A. Wilhelms. 1967. The continuous determination of low level iron, soluble phosphate and total phosphate with the AutoAnalyzer. Technicon Symp. 1:385-89.
Methods

Coble, P. G. (1996). Characterization of marine and terrestrial DOM in seawater using excitation-emission matrix spectroscopy. Marine Chemistry, 51(4), 325–346. doi:10.1016/0304-4203(95)00062-3
Methods

Gordon, L. I., J. C. Jennings, JR, A. A. Ross, and J. M. Krest. (1994). A suggested protocol for continuous flow analysis of seawater nutrients (phosphate, nitrate, nitrite, and silicic acid) in the WOCE Hydrographic Program and the Joint Global Ocean Fluxes Study. WHP Office Report 91-1. Revision 1, Nov. 1994. WOCE Hydrographic Program Office, Woods Hole, MA.
Methods

Nelson, C. E., Donahue, M. J., Dulaiova, H., Goldberg, S. J., La Valle, F. F., Lubarsky, K., … Thomas, F. I. M. (2015). Fluorescent dissolved organic matter as a multivariate biogeochemical tracer of submarine groundwater discharge in coral reef ecosystems. Marine Chemistry, 177, 232–243. doi:10.1016/j.marchem.2015.06.026
Methods

Patton, C. J. (1983) Design, characterization and applications of a miniature continuous flow analysis system. Ph.D. Thesis, Mich. State Univ. U. Microfilms International, Ann Arbor, Mich. 150 pp.
Methods

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Parameters

Parameter	Description	Units

Sample_ID	Unique sample identity	unitless
Type	Abbreviation for sampled sponge species, first capitalized letter representing the first letter of the genus name, last three letters representing the first three of the species name. E.g Ndig = N. digitalis	unitless
Species	Inhalant water samples from outside of sponge or exhalent water samples leaving the osculum of the sponge after it has been filtered by the sponge.	unitless
Sample	Sample replicate number (1-10)	unitless
Microbial_Abundance	High Microbial Abundance (HMA) or Low Microbial Abundance (LMA)	unitless
Microbial_Community_Composition	The microbial community composition was defined by the presence of both nitrifying bacteria and archaea and photoautotrophs ("both"), nitrifiers only ("nitrate"), or neither of these microbial groups ("neither") (data from Freeman et al., 2020).	unitless
DayCollected	Day of experiment run in which sample was collected.	unitless
Row	Row number within the artificial reef in which the sponge that the samples were taken from was located.	unitless
Neighbor	The sponge species neighboring the individual that was sampled.	unitless
Total_sample_RU_after_blank_subtraction	Total Raman units in the sample minus the Raman units of the milliQ water blank	Raman units of water (RU)
M_to_C_ratio	Ratio of Coble Peak M (Marine Humic-like) to Cole Peak C (Visible Humic-like)	unitless
BIX	Biological Index meaning the tolerance values assigned to species.	biotic index score
HIX	Jumification index indicates the ratio between the humified and the non-humified portion of an organic substance	unitless
FI	Fluorescence Index used to distinguish a microbial (FI > 1.8) or terrestrial (FI < 1.2) source	unitless
Ultra_Violet_Humic_like	Coble Peak A (Ultra Violet Humic-like)	Raman units of water (RU)
Marine_Humic_like	Coble Peak M (Marine Humic-like)	Raman units of water (RU)
Visible_Humic_like	Coble Peak C (Visible Humic-like)	Raman units of water (RU)
Tryptophan_like	Coble Beak T (Tryptophan-like)	Raman units of water (RU)
Tyrosine_like	Coble Peak B (Tyrosine-like)	Raman units of water (RU)
Phenylalanine_like	Coble Peak F (Phenylalanine-like)	Raman units of water (RU)

Fulvic_Acid_like	Peak D (Stedmon, 2003)	Raman units of water (RU)
Lignin_like	Peaks corresponding to lignin phenols as in Herenes et al. 2009	Raman units of water (RU)
Phosphate	Concentration of dissolved phosphate in micromoles per liter	micromoles per liter of water (umol/L)
Nitrate_Nitrite	Concentration of dissolved nitrate that was reduced to nitrite plus the nitrite in micromoles per liter	micromoles per liter of water (umol/L)
Silicate	Concentration of dissolved silicate in micromoles per liter	micromoles per liter of water (umol/L)
Nitrite	Concentration of dissolved nitrite in micromoles per liter	micromoles per liter of water (umol/L)
Ammonium	Concentration of dissolved ammonium in micromoles per liter	micromoles per liter of water (umol/L)
Filter_Initial_weight	Dry weight of the GF/F filter	milligrams (mg)
Filter_Post_weight	Weight of the GF/F filter after water filtration and drying at 60 degrees C overnight	milligrams (mg)
Difference_Filter_in_weight	The difference between the weight of the GF/F filter after water filtration and the dry weight of the filter	milligrams (mg)
Filter_Total_pcnt_N	Total nitrogen on the GF/F filter as a percentage	percent
Filter_Total_N	Total nitrogen in mg on the GF/F filter based on percent nitrogen and the difference in filter weight	milligrams (mg)
Filter_Total_pcnt_C	Total carbon on the GF/F filter as a percentage	percent
Filter_Total_C	Total carbon in mg on the GF/F filter based on percent nitrogen and the difference in filter weight	milligrams (mg)
Filter_C_to_N_Ratio	Ratio of the percent C to percent N on the GF/F filters	unitless

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Instruments

Dataset-specific Instrument Name	Alpkem RFA 300
Generic Instrument Name	Alpkem RFA300
Dataset-specific Description	Alpkem RFA 300™ components were used to measure silicic acid, nitrate plus nitrite, and nitrite.
Generic Instrument Description	A rapid flow analyser (RFA) that may be used to measure nutrient concentrations in seawater. It is an air-segmented, continuous flow instrument comprising a sampler, a peristaltic pump which simultaneously pumps samples, reagents and air bubbles through the system, analytical cartridge, heating bath, colorimeter, data station, and printer. The RFA-300 was a precursor to the smaller Alpkem RFA/2 (also RFA II or RFA-2).

Dataset-specific Instrument Name	Thermo Flash 1000
Generic Instrument Name	Elemental Analyzer
Dataset-specific Description	A Thermo Flash 1000 series elemental analyzer was used for elemental analysis of GF/F filters.
Generic Instrument Description	Instruments that quantify carbon, nitrogen and sometimes other elements by combusting the sample at very high temperature and assaying the resulting gaseous oxides. Usually used for samples including organic material.

Dataset-specific Instrument Name	Horiba Aqualog scanning fluorometer
Generic Instrument Name	Fluorometer
Generic Instrument Description	A fluorometer or fluorimeter is a device used to measure parameters of fluorescence: its intensity and wavelength distribution of emission spectrum after excitation by a certain spectrum of light. The instrument is designed to measure the amount of stimulated electromagnetic radiation produced by pulses of electromagnetic radiation emitted into a water sample or in situ.

Dataset-specific Instrument Name	Technicon AutoAnalyzer II
Generic Instrument Name	Technicon AutoAnalyzer II
Dataset-specific Description	Technicon AutoAnalyzer II™ components were used to measure phosphate and ammonium.
Generic Instrument Description	A rapid flow analyzer that may be used to measure nutrient concentrations in seawater. It is a continuous segmented flow instrument consisting of a sampler, peristaltic pump, analytical cartridge, heating bath, and colorimeter. See more information about this instrument from the manufacturer.

Dataset-specific Instrument Name	Shimadzu TOC-5000A
Generic Instrument Name	Total Organic Carbon Analyzer
Dataset-specific Description	Samples for dissolved organic carbon (DOC) and total dissolved nitrogen (TDN) were sent to the UGA Stable Isotope Ecology Laboratory for analysis using a Shimadzu TOC-5000A Total Organic Carbon Analyzer.
Generic Instrument Description	A unit that accurately determines the carbon concentrations of organic compounds typically by detecting and measuring its combustion product (CO2). See description document at: http://bcodata.whoi.edu/LaurentianGreatLakes_Chemistry/bs116.pdf

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Project Information

Collaborative Research: Investigations into microbially mediated ecological diversification in sponges (Ecological Diversification in Sponges)

Coverage: Caribbean coast of Panama

NSF Award Abstract:
Coral reefs represent a paradox because, despite their immense productivity and biodiversity, they are found in nutrient-poor habitats that are equivalent to "marine deserts." High biodiversity is often associated with a division of resources that allows many types of organisms to coexist with minimal competition. Indeed, unlike many other organisms on coral reefs, sponges are adapted to efficiently remove bacteria, phytoplankton, and dissolved organic matter from seawater by filter-feeding. Sponges are a dominant component of coral reefs worldwide and in the Caribbean, where their biomass exceeds that of reef-building corals. For almost a quarter century, the success of sponges in the Caribbean has been linked to their filter-feeding ability. However, recent work demonstrated that coexisting sponges on Caribbean reefs host unique communities of bacteria that might allow sponges to access multiple pools of nutrients that are not available to other organisms. In this project, the investigators will test the hypothesis that ecologically dominant sponge species in the Caribbean have unique metabolic strategies that are mediated by their associations with microbes that live within the sponge body. This research will combine manipulative field experiments with a novel combination of modern analytical tools to investigate both filter-feeding by sponge hosts and the metabolic pathways of their microbes. This work will advance our understanding of the ecological and evolutionary forces that have helped shape the species present on Caribbean coral reefs. Additionally, this project will support three early-career investigators and provide training opportunities for graduate and undergraduate students at Nova Southeastern University, Appalachian State University, Stony Brook University, and Smithsonian Marine Station.

The investigators will also develop innovative outreach programs that expand existing platforms at their institutions to increase public engagement and scientific literacy.

Marine sponges have been widely successful in their expansion across ecological niches in the Caribbean, with biomass often exceeding that of reef-building corals and high species diversity. However, whether this success is linked to efficient heterotrophic filter-feeding on organic carbon in the water column or to their evolutionary investment in microbial symbionts is yet to be fully elucidated. Microbial symbionts expand the metabolic capabilities of host sponges, supplementing heterotrophic feeding with inorganic carbon and nitrogen, mediating the assimilation of dissolved organic matter, and facilitating recycling of host-derived nitrogen. Despite these benefits, microbial symbiont communities are widely divergent across coexisting sponge species and there is substantial variation in host reliance on symbiont-derived carbon and nitrogen among host sponges; therefore, these associations likely mediate the ecological diversification of coexisting sponge species. The goal of this project is to test this transformative hypothesis by adopting an integrative approach to assess the individual components of holobiont metabolism (i.e., microbial symbionts and sponge host) in ten of the most common sponge species in the Caribbean. The investigators will isolate autotrophic and heterotrophic metabolic pathways and explore potential links between microbial symbiont community composition and the assimilation of particulate and dissolved organic matter (POM and DOM) from seawater. This project will elucidate whether Caribbean sponge species are on similar or divergent evolutionary trajectories, and will provide information that is critical for our understanding of how conditions in the Caribbean basin have shaped the evolution of benthic organisms.

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Funding

Funding Source	Award
NSF Division of Ocean Sciences (NSF OCE)	OCE-1915949
NSF Division of Ocean Sciences (NSF OCE)	OCE-1756799
NSF Division of Ocean Sciences (NSF OCE)	OCE-1929293
NSF Division of Ocean Sciences (NSF OCE)	OCE-1756114
NSF Division of Ocean Sciences (NSF OCE)	OCE-1756249
NSF Division of Ocean Sciences (NSF OCE)	OCE-1756171

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